

jc662 U.S. PTO
05/25/99

APPLICATION FOR UNITED STATES PATENT

in the name of

Keith A. Kretz

and

Jay M. Short

of

Diversa Corporation

for

RECOMBINANT BACTERIAL PHYTASES AND USES THEREOF

jc542 U.S. PTO
09/318528
05/25/99

I hereby certify under 37 CFR 1.10 that this correspondence is being deposited with the United States Postal Service as **Express Mail Post Office To Addressee** with sufficient postage on the date indicated below and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

signature

Gregory H. Shen

name

DATE OF DEPOSIT: 05/25/99

EXPRESS MAIL NO.: EM EE090620416 US

RECOMBINANT BACTERIAL PHYTASES AND USES THEREOF

09/291,931
This application is a continuation-in-part of U.S. Patent Application Serial No. ~~Not yet~~ ^{pending} assigned, entitled "Novel Phytase", filed April 13, 1999, which is hereby incorporated by reference in its entirety, which is a continuation of U.S. Patent Application Serial No. 09/259,214, filed March 1, 1999, ~~pending~~ ^{now U.S. Pat. No. 6,110,719} which is hereby incorporated by reference in its entirety, which is a divisional of U.S. Patent Application Serial No. 08/910,798 (entitled "Phytase"), now U.S. Patent No. 5,876,997, filed August 13, 1997, which is hereby incorporated by reference in its entirety.

CONTENTS

1. FIELD OF THE INVENTION
2. BACKGROUND
 - 2.1 - General Overview of the Problem to Be Solved
 - 2.1.1 - Brief Summary
 - 2.1.2 - Nutritional Concerns
 - 2.1.3 - Ex Vivo Processing Concerns
 - 2.1.4 - Medical Concerns
 - 2.1.5 - Environmental Concerns
 - 2.1.6 - Financial Concerns
 - 2.2 - General Overview of Phytate & Phytate Hydrolysis
 - 2.2.1 - Phytate Hydrolysis Leads to Release of Nutrients
 - 2.2.2 - Microbial Enzymes Can Hydrolyze Phytate
 - 2.3 - Solving the Problem of Insufficient Phytate Hydrolysis
 - 2.3.1 - Enzyme Additives in Commercial Applications
 - 2.3.2 - Optimization of Enzyme Additives Is Needed
3. SUMMARY OF THE INVENTION
4. BRIEF DESCRIPTION OF THE DRAWINGS
5. DEFINITIONS OF TERMS

6. DETAILED DESCRIPTION OF THE INVENTION

6.1 - Novel Phytase

- 6.1.1 - General Overview**
- 6.1.2 - Phytase Polypeptides**
- 6.1.3 - Phytase Polynucleotides**
- 6.1.4 - Methods of Isolation**
- 6.1.5 - Determination of Activity**

6.2 - Production Of Novel Phytase

- 6.2.1 - General Overview**
- 6.2.2 - Recombinant Expression**
- 6.2.3 - Use Of Transgenic Plants And Plant Organs**
- 6.2.4 - Examples of Serviceable Plants**
- 6.2.5 - Plant Transformation Methods**
- 6.2.6 - Methods for Dicots**
- 6.2.7 - Methods for Monocots**
- 6.2.8 - Methods for Expression in Plants**
- 6.2.9 - Dual Expression of Novel Phytase & Other Molecules**
- 6.2.10 - Soluble Preparation of Novel Phytase & Stabilized Liquid Formulations Thereof**

6.3 - Use Of Novel Phytase

- 6.3.1 - General Overview**
- 6.3.2 - Administration to Organisms**
- 6.3.3 - Steeping Of Cereals**
- 6.3.4 - Preparation Of Bread Dough**
- 6.3.5 - Production Of Soybean-Containing Foodstuffs**
- 6.3.6 - Production Of Liquid Foodstuffs Including Sake**
- 6.3.7 - Production Of Mineral Absorbefacient**
- 6.3.8 - Use In Combination With Other Phytases &/Or Acid Phosphatases**
- 6.3.9 - Use In Combination With Enzymes That Act On Polysaccharides (e.g. Xylanases)**

- 6.3.10 - Use In Combination With Vitamin D**
- 6.3.11 - Use In Combination With Lactic Acid-Producing Bacteria**
- 6.3.12 - Solubilization Of Proteins In Combination With Proteases**
- 6.3.13 - Triple Treatment Of Compost Using Novel Phytase, Saponin, & Chitosan**
- 6.3.14 - Use As Hybridization Probes & Amplification Templates**
- 6.3.15 - Use in Directed Evolution**
- 6.3.16 - Use in antibody production**

EXAMPLE 1 - Isolation, Bacterial Expression And Purification Of Phytase